BIOETHANOL PRODUCTION FROM TACCA LEONTOPETHALOIDES STARCH BY CO-CULTURING OF RAGI TAPAI WITH SACCHAROMYCES CEREVISIAE IN A STIRRED BIOREACTOR TANK

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***Abstract*- Bioethanol can be produced from fermentation of *Tacca leontopethaloides* starch by culturing or co-culturing *Saccharomyces cerevisiae* and ragi tapai in bioreator. The comparison study between different cultures for fermentation was done which were single culture of *S.cerevisiae*, and co-culturing of *S.cerevisiae* and Ragi tapai. The results show that for single culture, the production of ethanol was averages at 0.2774 g/l which is lower than co-culture which is averages at 0.2805 due to co-culture has better condition to produce more ethanol effectively. The fermentation had been done for 72 hours and the sample was taken every 2 hours for 12 hours and every 6 hours for 60 hours. The total 16 samples was collected throughout the experiment. Glucose content, ethanol and cell dry weight analysis were used in this experiment to observe the production of ethanol.**

Keywords— fermentation, Tacca leontopethaloides starch, ragi tapai, Saccharomyces cerevisiae, ethanol, co-culture.

I. INTRODUCTION

Nowadays, people depends on gasoline without noticing the fossil fuel source reserve are depleting every day. This will absolutely will make fuel prices increase and at the same time unfolding energy crisis [1]. Therefore, some alternatives are needed to avoid these problems. Production of ecologically sustainable bio-fuels is gaining attentions from all over the world [2]. One of the famous sustainable bio-fuels is bioethanol which can be produced from fermentation by using renewable resources. The renewable resources comes from starch, glucose and cellulose. The most common source used for fermentation is from starch.

There are many types of starches can be used to produce ethanol such as potato, sweet potato, yam, arrowroot and cassava. [1]. *Tacca leontopetaloids* or commonly called Polynesian arrowroot starch can be easily found growing from western Africa through southern Asia to northern Australia [3]. This plant also can be found abundant in Malaysia, as the condition here is optimum for its growth. Furthermore, this plans also one of the plants that can be used to produce ethanol. In addition, there are low utility of this plant due to it bitter taste, therefore, it is one of the good choice to produce bioethanol as it will not affected the food source. There are also not very much research to produce ethanol from this plant.

In production of ethanol, the standard method is multistage fermentation strategy by using amylase enzymes for liquefaction and saccharification followed by fermentation with yeast or using amylolytic yeast as an alternative [4], [5]. Nevertheless, there are not many amylolytic yeasts able to efficiently hydrolyze starch [4], [6]. To overcome this setback, ragi tapai can serves as an alternative [4]. Ragi tapai is a dry-starter culture that is made from a mixture of rice flour, water and spices or sugar cane extract [4]

In this research, bioethanol will be produced by fermentation of *T. leontopetaloides* by co-culturing ragi tapai and *Saccharomyces cerevisiae* in stirred tank bioreactor with another objective to compare fermentation profile between different cultures.

II. METHODOLOGY

A. Materials

Yeast strains, co-culture and starch

The starch chosen were *tacca leontapethaloids* starch for sugar production that was used by the yeast. The yeast strain used for the research are *Saccharomyces cerevisiae* (industrial yeast) that is obtainable from supermarket nearby. Yeast were used to produce ethanol, carbon dioxide and etc. The co-culture used were ragi tapai that is obtainable from local store near Klang. Ragi tapai was used due to it contain necessary nutrient for amylolytic process, which obtained from fresh market. Amylolytic process is the process of converting starch to sugar [7].

B. Methods

Fermentation in bioreactor

Fermentation in bioreactor was where the bioethanol will be produced. Firstly, 10% *Tacca leonpethaloids* flour was mixed in 60°C preheated water in a fermenter. Then, the temperature was raised to 70°C and mixed for 1 hour. Next, the temperature was maintained at 30°C to be inoculated with firstly with Ragi Tapai, next, with Co-cultured ragi tapai and *Saccharomyces cerevisiae* at zero hour and last but not least, after 1 hour, ragi tapai was added followed by *Saccharomyces cerevisiae*.[8]. The agitation was maintained at 200 RPM.

C. Analytical method

The sample were collected for 72 hours, which every 2 hours for 12 hours and every 6 hours for 60 hours. So there were 16 sample collected in the experiment. The sample were centrifuged at 5000 rpm for 30 minutes and the supernatant were analyzed. Glucose was analyzed by adding 2mL of DNSA reagent was added samples. Next, the tube was vortexed, then heated at 100 ºC for 15-20 minutes and cooled down with tap water. After that, the solutions were put into cuvette and absorbance was recorded at 540 nm [9]. Ethanol was analyzed by using spectrophotometer. Supernatant was distillated using a rotary evaporator. The distillate was used to determine bioethanol concentration calorimetrically using

potassium dichromate method [10]. A test tube containing 10 mL of acidic potassium dichromate reagent was added with 2 mL of distillated sample and mixed well. The tubes were closed with Para film and kept in a water bath at 60 ºC for 20 min then, it was cooled to room temperature. The absorption of the reaction mixture was measured at 600 nm by a spectrophotometer. Blank consisted of 2 mL of distilled water mixed with 10 mL of potassium dichromate acidic reagent. This reagent was prepared by dissolving 34 g of potassium dichromate in 400 mL of distilled water with 325 mL of sulfuric acid and by making up the volume to 1 L. A standard curve was prepared under similar conditions using standard solutions of ethanol in distilled water [11].

III. RESULTS AND DISCUSSION

A. *Fermentation profiles*

The result was obtained from some analysis that was made after the fermentation was done. The supernatant were used to analyze glucose and ethanol concentration while the residue was weight after left in an oven overnight. These are the result that have been plotted after analyzed.

a) Single culture

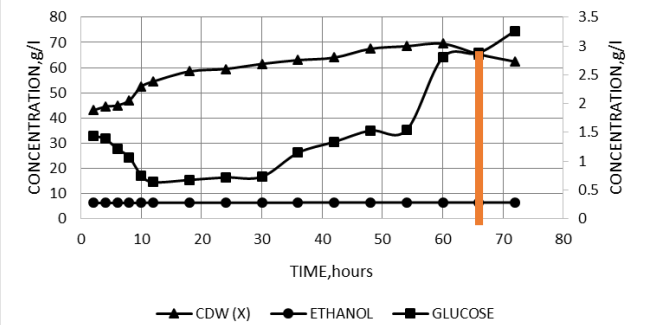

Figure 1: Fermentation profile for single culture

b) Co-culture

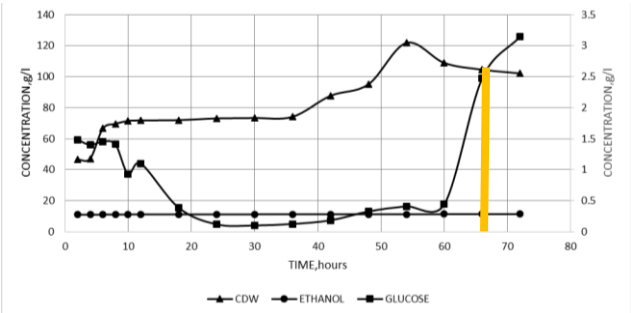

Figure 2: Fermentation profile for co-culture

Table 1: Comparison between single culture and co-culture on ethanol production

Cultures	Ethanol concentration, g/l
Single	0.2774
Co-culture	0.2805

B. *Exponential growth*

(1) Single culture

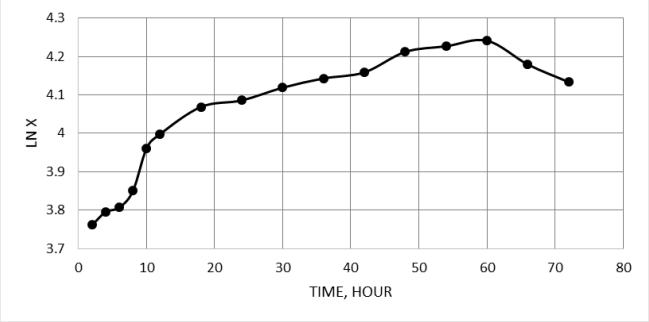

Figure 3: Exponential CDW over time for single culture

(2) Co-culture

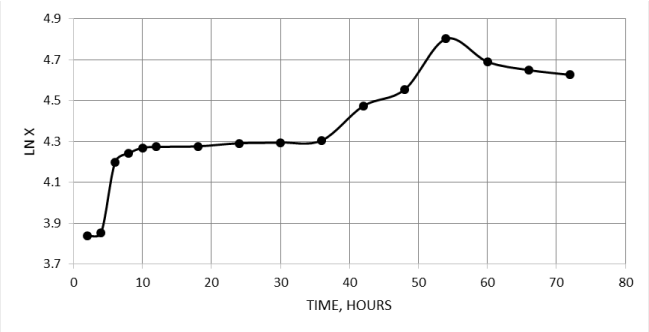

Figure 4: Exponential CDW over time for co-culture

C. *Yield*

Yield is obtainable from gradient of the graph.

(1) Substrate Yield

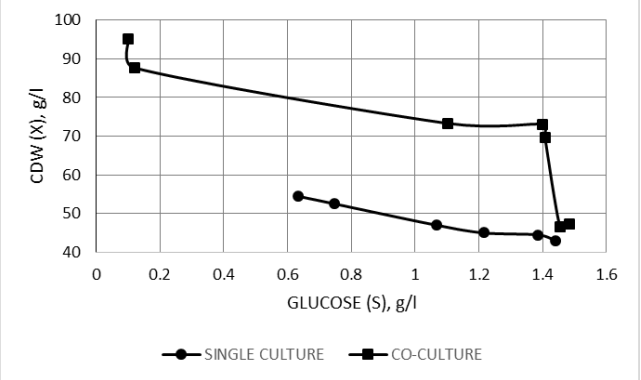

Figure 5: Apparent yield for substrate

(2) Product Yield

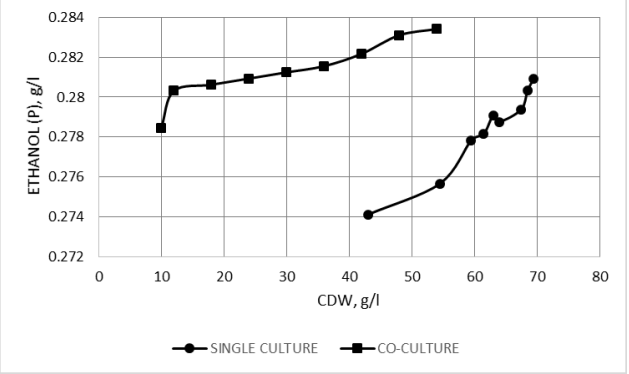

Figure 6: Product yield for both cultures

Table 2: Comparison of apparent yields, product yield and maximum growth rate.

Cultures	Apparent Yield, g(cell/g substrate)	Product Yield, g(product/g cell)	Maximum growth rate, μmax
Single	14.212	3884.177	0.00642
Co-culture	19.365	8867.650	0.01850

The fermentation was done by co-culturing ragi tapai and *S. cerevisiae* for co-culture and *S. cerevisiae* only for single culture. *T. leontopetaloides* starch had been used to provide glucose for the culture. .It had been done for 72 hours and the sample was taken every 2 hours for 12 hours and every 6 hours for 60 hours. The total 16 samples was collected throughout the experiment. Glucose content, ethanol and cell dry weight analysis were used in this experiment to observe the production of ethanol.

The fermentation profile consist of ethanol, glucose and cell dry weight are shown in Figure 1 and Figure 2. For the glucose analysis, based on Figure 1 and Figure 2 show that the glucose concentrations was already low from the beginning with 1.4 g/l for both culture and getting lower to 0.7g/l for single culture 0.14 g/l for co-culture but increasing drastically at the end by about 3.265 g/l and 3.147 g/l respectively. The reason why the glucose is very low at the beginning is due to no or less enzyme were present to hydrolyzed the starch to glucose [1]. The glucose become lower at the middle of fermentation due to the culture consuming the glucose [4]. Towards the end, where the cell undergoes death phase, the concentration of glucose suddenly become high due to amylase produced by ragi still converting starch to reducing sugar but it is not consumed by the cultures [12]. In addition, the *S. cerevisiae* also produced and release amylase in fermentation [13]. These clearly proved that the high glucose content in the end due to presence of amylase that hydrolyzed the starch.

Based on Figure 1 and Figure 2 also show that ethanol production which were averages 0.2774 g/l and 0.2805g/l respectively as tabulated in Table 1. This show that ethanol produced from co-culture was slightly higher than single culture. This result is acceptable according to [14] which stated fermentation by co-culture will gave higher production of ethanol. One of the co-culture advantages that made it is better culture due to presence of other microorganisms at the beginning of fermentation help to add lacking nutrients in the medium [15]. The ethanol production also show constant reading towards the end of fermentation for both culture due to death phase occurred for both cell in the cultures, so there were no more activity there [16]. The production also very low due to this is a small scale production. In addition, Figure 1 and Figure 2 also show that the fermentations ended almost at the same time which at about 66 to 68 hours. Therefore, it is suggested to do experiment about 68 hours to get better final results.

Exponential growth analysis based on Figure 3 and Figure 4 show that the cells undergo few phases which were lag phase at the start, followed by exponential phases, then it went through stationary phase for the longest time and finally death phase at the end. In the lag phase, the cell in the culture started to divide slowly before exponential phase where they divided actively [17]. At the stationary phase, the number of cell died was equal to number of cell reproduced and at the end phases is where all the cell died [16]. Therefore both culture show normal lifespan of the cell [16]. For co-culture, the death phases showed as early at 54 hours. This is due to the pH controller for bioreactor was not functioning properly at that time. this problem lead to acidic condition which were confirmed by [18] that growth rate *S. cerevisiae* decreasing when pH was decreased from 3.5 to 3.0. From Figure 3 and 4, maximum specific growth rate also can be calculated from gradient where exponential growth take place [16]. It also can be calculated from Equation 1 [19]. Maximum specific growth rate for single culture and co-culture is 0.00642 and 0.01850 respectively which also tabulated in table 2. This clearly show that maximum growth rate for co-culture is better due to *S. cerevisiae* and ragi tapai complimented each other for lack of nutrient [15]. The ragi also provide an alternative for amylolytic process [8], which is the process of converting starch to sugar [7]. Therefore the starch can hydrolyzed faster.

$$\mu_m = \frac{lnX_0 - lnX}{t_2 - t_1} \qquad \text{(Equation 1)}$$

In addition, based Figure 5 shows the graph of cell dry weight over glucose concentration. The graph shows that it had negative slope due to substrate was consumed and decreasing [20]. Ethanol apparent yield was obtained from the gradient of the graph. The result obtained were 19.365 g (cell/g substrate) for co-culture. This was 27% higher than the yield obtained with single culture of *S. cerevisiae* at 14.212 g (cell/g substrate) as tabulated in Table 2. Next, based on figure 6, it show the graph of ethanol against cell dry weight. It shows that ethanol formation increase as cell dry weight increase and had positive slope. The product yield was obtained from gradient of the graph in figure 6 [20]. The results show that ethanol produced from co-culture was 8867.650 g(product/g cell) which gave rise to 56% higher yield compared to single culture with 3884.177 g(product/g cell). Both results also show that, co-culture had higher yield than single culture as Nuwamanya [21] stated, ethanol yield using co-culture is better than pure culture.

IV. Conclusion

.In this research, ethanol production from different culture in bioreactor had been investigated. The objectives ware achieved as the culture used were single culture of *S. cerevisiae* and co-culture of *S. cerevisiae* with ragi tapai. These cultures gave different reading in all analysis as co-culture was proven better as it had 56% more product yield and 27% higher apparent yield than single culture. It is also proven direct fermentation of *T. leontopetaloides* starch was feasible with both cultures and could be new feedstock for the process.

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VI. REFERENCES

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